Penifulvins B−E and a Silphinene Analogue: Sesquiterpenoids from a Fungicolous Isolate of *Penicillium griseofulvum*

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Penifulvins B−E (**2**−**5**), four new sesquiterpenoids with a dioxa[5.5.5.6]fenestrane ring system, have been isolated from cultures of an isolate of *Penicillium griseofulvum* (NRRL 35584), together with a new silphinene derivative, 12-hydroxysilphinene-15-oic acid (**6**). Penifulvins B−E (**2**−**5**) are oxidized analogues of penifulvin A (**1**) and were identified by analysis of NMR and MS data. 12-Hydroxysilphinen-15-oic acid (**6**) is biogenetically similar, and penifulvins A−E are presumed to be derived from a silphinene precursor. The structures of **2**−**6**, including absolute configuration, were assigned by analysis of NMR data and application of chemical methods.

Mycoparasitic and fungicolous fungi are those that parasitize or colonize the hyphae or survival structures of other species.[1,2] Our studies of mycoparasitic and fungicolous fungi have led to the isolation of a variety of new bioactive secondary metabolites.[3−8] In the course of this project, a Hawaiian isolate of *Penicillium griseofulvum* Dierckx (MYC-1728 = NRRL 35584) was subjected to chemical investigation. An organic extract from cultures of *P. griseofulvum* NRRL 35584 showed potent antifungal and antiinsectan activity in preliminary assays. A sesquiterpenoid with a novel ring system (penifulvin A; **1**) was initially isolated as the major component, together with the known antifungal compound mycophenolic acid.[3] Continued studies of this extract have led to the identification of four additional analogues (penifulvins B−E; **2**−**5**) and a new silphinene derivative (12-hydroxysilphinene-15-oic acid; **6**), together with the known *Penicillium* metabolites asperphenamate, brevianamide A, brevianamide E, 1-deoxypebrolide, and desacetylpebrolide. Penifulvins B−E (**2**−**5**) are oxidized analogues of **1**, while 12-hydroxysilphinen-15-oic acid (**6**) is a biogenetically related metabolite with a different ring system. This report describes the isolation and structure elucidation of compounds **2**−**6**.

Results and Discussion

Penifulvins A−E (**1**−**5**) and 12-hydroxysilphinen-15-oic acid (**6**) were all obtained by processes involving silica gel column chromatography followed by reversed-phase HPLC. HRESIMS and NMR data established the molecular formula of penifulvin B (**2**) as $C_{15}H_{20}O_5$. This formula contains one more oxygen atom than that of penifulvin A (**1**). Analysis of ^1H and ^{13}C NMR data for **2** (Tables 1 and 2) revealed considerable structural similarities to penifulvin A (**1**), which facilitated the structure elucidation of this metabolite. Characteristic resonances for the dioxa[5.5.5.6]fenestrane system found in **1**, including the diagnostic acetal (δ_H 5.97; δ_C 103.9) and central quaternary carbon (δ_C 66.7) signals, were observed. ^1H, ^{13}C, and DEPT NMR data indicated that **2** has two methyl groups, rather than three as in penifulvin A, and has an oxygenated methylene unit instead (δ_H 3.45 and 3.48; δ_C 72.5), suggesting that one of the three methyl groups in **1** is oxidized to a primary alcohol in **2**. This conclusion was confirmed and the OH group was located by analysis of HMBC and NOESY data. The oxymethylene proton resonances showed HMBC correlations with one methyl carbon at δ_C 22.8, a quaternary carbon at δ_C 45.9, a methylene carbon at δ_C 53.9, and a methine carbon at δ_C 56.2, indicating that one of the geminal methyls at C-6 in **1** is replaced
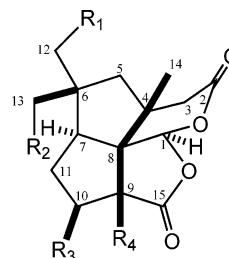
1	R_1 = H R_2 = H R_3 = H R_4 = H	
2	R_1 = OH R_2 = H R_3 = H R_4 = H	
3	R_1 = H R_2 = OH R_3 = H R_4 = H	
4	R_1 = H R_2 = H R_3 = H R_4 = OH	
5	R_1 = H R_2 = H R_3 = OH R_4 = H	

6
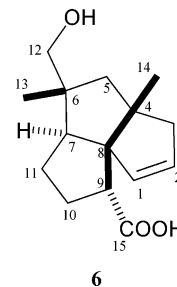
by a CH_2OH group in **2**. Chemical shift changes at C-5, C-6, C-7, and C-13 relative to the corresponding data for **1** were consistent with addition of an OH group at this position. NOESY correlations of the oxymethylene signals with H-7 (δ_H 2.59) and H-5α (δ_H 2.07) indicated that the hydroxymethylene unit has the α-orientation. Thus, the structure of penifulvin B was determined as shown in **2**.

HRESIMS and NMR data indicated that penifulvin C (**3**) is an isomer of penifulvin B (**2**). The close resemblance of the ^1H and ^{13}C NMR data for **3** to those of **2** (Tables 1 and 2) indicated the presence of the same skeleton found in **1** and **2** and suggested that the only difference is that C-13 bears the OH group, rather than C-12, as in **2**. This conclusion was confirmed by analysis of HMBC and NOESY data. HMBC correlations from the oxymethylene signals at δ_H 3.45 and 3.49 matched those of the oxymethylene signals in **2**, but in this instance, the CH_2O protons showed NOESY correlations to H_2-11, while the methyl singlet (CH_3-12) showed correlations to H-1, H-7, and H-3α.

The molecular formula of penifulvin D (**4**) was also established as $C_{15}H_{20}O_5$ on the basis of HRESIMS and NMR data and again showed clear similarities to **1**−**3**. In this instance, a broad

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Table 1. ¹H NMR Data for Penifulvins B−E (**2**−**5**) and 12-Hydroxysilphinen-15-oic Acid (**6**)[a]

H#	2 $\delta_H{}^a$ (mult., J in Hz)	3 $\delta_H{}^a$ (mult., J in Hz)	4 $\delta_H{}^a$ (mult., J in Hz)	5 $\delta_H{}^a$ (mult., J in Hz)	6 $\delta_H{}^a$ (mult., J in Hz)
1	5.97 (s)	5.95 (s)	5.92 (s)	5.91 (s)	5.56 (dt, 2.0, 6.0)
2					5.51 (dt, 2.0, 6.0)
3α	3.17 (d, 15)	2.81 (d, 15)	2.65 (d, 15)	2.83 (d, 15)	2.34 (dt, 2.0, 17)
3β	2.32 (d, 15)	2.48 (d, 15)	2.43 (d, 15)	2.46 (d, 15)	2.26 (dt, 2.0, 17)
5α	2.07 (d, 14)	1.73 (d, 14)[b]	1.72 (d, 14)	1.69 (d, 14)	1.91 (d, 14)
5β	1.61 (d, 14)	1.67 (d, 14)[b]	2.02 (d, 14)	2.07 (d, 14)	1.57 (d, 14)
7	2.59 (dd, 3.0, 9.8)	2.39 (dd, 4.4, 9.6)	2.44 (dd, 5.4, 9.6)	2.32 (dd, 6.4, 10)	2.12 (dd, 8.0, 11)
9	2.96 (d, 6.6)	2.96 (dd, 2.4, 8.4)		3.06 (br s)	
10α	2.33 (ddt, 2.0, 7.2, 13)	2.24 (m)	2.41 (dt, 5.4, 14)	4.66 (ddd, 1.6, 3.6, 6.0)	1.98 (m)
10β	1.84 (m)	1.92 (m)	2.12 (ddd, 5.4, 10, 14)		
11α	1.61 (m)	1.78 (m)	1.80 (dtd, 6.6, 9.6, 14)	2.07 (ddd, 6.0, 10, 15)	1.72 (m)
11β	1.92 (m)	1.92 (m)	2.01 (ddd, 5.4, 10, 18)	1.89 (dddd, 1.6, 3.6, 6.0, 15)	1.37 (m)
12	3.45 (d, 10); 3.48 (d, 10)	1.30 (s)	1.18 (s)	1.25 (s)	3.35 (d, 10); 3.37 (d, 10)
13	0.98 (s)	3.45 (d, 11); 3.49 (d, 11)	1.09 (s)	1.05 (s)	0.99 (s)
14	1.10 (s)	1.10 (s)	1.34 (s)	1.19 (s)	1.27 (s)
OH			2.55 (s)		

[a] Data were recorded in CDCl₃ at 600 MHz. [b]These assignments are interchangeable.

**Table 2.** ¹³C NMR Data for Penifulvins B−E (**2**−**5**) and 12-Hydroxysilphinen-15-oic Acid (**6**)[a]

C#	2	3	4	5	6
1	103.9	103.8	103.1	104.3	137.1
2	169.4	168.5	167.7	168.7	128.6
3	43.5	44.5	44.6	44.3	50.6
4	42.3	42.2	42.9	42.6	49.7
5	53.9	51.0	56.7	54.1	53.8
6	45.9	45.4	39.8	40.6	44.9
7	56.2	59.2	59.9	60.8	58.6
8	66.7	66.4	66.6	66.8	73.1
9	46.7	46.4	84.8	55.1	49.8
10	31.4	30.4	39.5	76.8	31.6
11	27.6	27.6	24.3	38.2	28.5
12	72.5	27.9	33.3	33.0	71.6
13	22.8	69.4	27.2	28.3	22.4
14	27.6	27.5	25.1	28.7	27.6
15	177.9	177.9	176.8	175.9	178.1

[a] Data were recorded in CDCl₃ at 100 MHz.

5a   R = (S)-MPA

5b   R = (R)-MPA

Figure 1. Observed chemical shift differences ($\Delta\delta = \delta_S - \delta_R$, ppm, 400 MHz) for the R- and S-MPA esters of penifulvin E (**5**).

exchangeable proton resonance was observed at δ_H 2.55, and ¹³C and DEPT NMR data for **4** suggested that the C-9 methine carbon found in **1**−**3** was oxidized to a tertiary alcohol (δ_C 84.8) in **4**. HMBC correlations from the OH signal to C-8, C-9, C-10, and C-15 and from H₂-10 to C-9, C-11, and C-15 confirmed attachment of the hydroxy group to C-9. The relative configuration was assigned by analysis of NOESY correlations, which matched well with those of penifulvins A−C (**1**−**3**), suggesting that the 9-OH group has the same (β) orientation as H-9 in **1**−**3**. Placement of the hydroxyl group at C-9 in an α-orientation would introduce considerable ring strain and would be expected to result in a quite different set of NOESY data.

HRESIMS and DEPT data for penifulvin E (**5**) established it as another isomer of penifulvins B−D. Its ¹H and ¹³C NMR data were again similar to those of **1**−**4**. In this case, signals for an additional oxygenated methine (δ_H 4.66; δ_C 76.8) appeared in the NMR spectra for **5**, implying that one of the methylene units present in **1** was oxidized to a secondary alcohol in **5**. HMBC correlations from the oxygenated methine proton to C-7, C-8, and C-15 indicated that the hydroxy group is attached to C-10. The relative configuration of the C-10 secondary alcohol moiety was assigned on the basis of NOESY data. In contrast to the assignment for **2**−**4**, the signal for H-11β, recognized by virtue of its NOESY correlation to H₃-13, was more upfield in the ¹H NMR spectrum of **5** than H-11α. Correlation of H-10 with H-7 and observation of a much stronger correlation of H-10 with H-11α than with H-11β enabled assignment of a β-orientation for the OH group.

The presence of a secondary alcohol moiety in **5** suggested that it might be suitable for stereochemical analysis using Mosher's method.[9] Treatment of **5** with (S)-MPAOH (α-methoxyphenylacetic
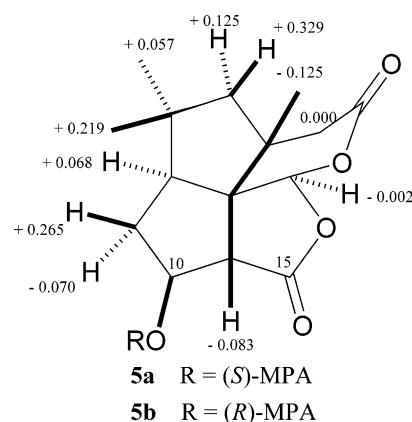
acid) or (R)-MPAOH in the presence of EDC [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide] and DMAP afforded the (S)-MPA ester (**5a**) or (R)-MPA ester (**5b**), respectively. Formation of the esters was confirmed in each case by a significant downfield shift of the H-10 resonance and the appearance of the expected aromatic and methoxy signals in the ¹H NMR spectrum. ¹H NMR signals for **5a** and **5b** were assigned by comparison with the data for **5** and were confirmed by analysis of NOESY correlations. Upon comparison of ¹H NMR chemical shifts for **5a** and **5b** ($\Delta\delta$ values shown in Figure 1), with one minor exception, all of the $\Delta\delta$ values observed for **5a** and **5b** (Figure 1) were consistent with assignment of the S-configuration at C-10, leading to the proposal of the overall absolute configuration as shown. Although the signal for H-11α showed a slight negative $\Delta\delta$ value, those for H-11β and other protons on the same side of the MPA group plane showed strongly positive $\Delta\delta$ values, particularly those on the same face of the ring system as the MPA group. Penifulvins A−D (**1**−**4**) are assumed to possess analogous absolute configurations.

The molecular formula for **6** was established as C₁₅H₂₂O₃ on the basis of HRESIMS, ¹³C NMR, and DEPT data. Although several similar structural features were evident, the pattern of NMR signals for **6** was quite different from those of **1**−**5**. Most notably, signals for a 1,2-disubstituted olefin and a single ester or acid carbonyl signal were present, while the acetal signals characteristic of **1**−**5** were not observed. The presence of one olefin unit and one carbonyl group indicated that **6** is tricyclic. The DEPT data in conjunction with the molecular formula indicated the presence of two exchangeable protons, which must be accounted for by hydroxy and carboxylic acid groups. ¹H and ¹³C NMR signals clearly indicated that the hydroxy group was part of an isolated hydroxymethylene unit. Analysis of COSY data revealed the same set of spin systems

Table 3. HMBC Data for Penifulvins B−E (**2**−**5**) and 12-Hydroxysilphinen-15-oic Acid (**6**)[a]

| C#	2 (H→C#)	3 (H→C#)	4 (H→C#)	5 (H→C#)	6 (H→C#)
1	7, 8, 9, 15	7, 8, 9, 15	2, 4, 7, 8, 9, 15	7, 8, 9, 15	2, 4, 8, 9
2					1, 4, 8
3α	2, 4, 5, 8, 14	2, 4, 5, 8, 14	2, 4, 5, 8, 14	2, 4, 5, 8, 14	1, 2, 4, 5, 14
3β	1[b], 2, 4, 5, 8, 14	1[b], 2, 4, 5, 8, 14	2, 4, 5, 8, 14	1[b], 2, 4, 5, 8, 14	1, 2, 4, 5, 14
5α	3, 4, 6, 7, 8, 12, 13, 14	3, 4, 7, 8, 13, 14	3, 4, 6, 7, 8, 12, 13, 14	3, 4, 6, 7, 8, 12	4, 6, 7, 8, 12, 13, 14
5β	2[b], 3, 4, 6, 7, 12, 13, 14	3, 4, 7, 8, 13, 14	2[b], 3, 4, 6, 7, 12, 13, 14	2[b], 3, 4, 6, 7, 12, 13, 14	4, 6, 7, 8, 12, 13, 14
7	4, 5, 6, 8, 10, 11, 12	1, 4, 6, 8, 10, 12	1, 5, 6, 12, 13	1, 4, 5, 6, 8, 10, 11, 12	1, 5, 6, 8, 9, 11, 12[b]
9	4, 7, 8, 10, 11, 15	4, 7, 10, 11, 15		4, 7, 8, 10, 11, 15	1[b], 4, 8, 10, 15
10α	7, 8, 9, 11, 15	7, 8, 9, 11	7, 9, 11, 15	7, 8, 15	7, 8, 9, 11, 15
10β	7, 9, 11, 15	7, 8, 9, 11	7, 9, 11, 15		7, 8, 9, 11, 15
11α	6, 7, 8, 9, 10	7, 8, 9, 10	7, 8, 9, 10	6, 7, 8, 9, 10	6, 7, 8, 9, 10
11β	6, 7, 8, 9, 10	7, 8, 9, 10	7, 8, 9, 10	6, 7, 8, 9, 10	6, 7, 8, 9, 10
12	5, 6, 7, 13	5, 6, 7, 13	5, 6, 7, 13	5, 6, 7, 13	5, 6, 7, 13
13	6, 7, 11[b], 12, 15	5, 6, 7, 12	5, 6, 7, 12	5, 6, 7, 12	5, 6, 7, 12
14	3, 4, 5, 6[b], 8, 9[b]	3, 4, 5, 8	3, 4, 5, 8	3, 4, 5, 8	3, 4, 5, 8
OH			8, 9, 10, 15		

[a] Data were recorded using CDCl₃ solutions at 600 MHz. [b] Four-bond correlations, most of which were of relatively low intensity.

6a   R = (*R*)-PGME

6b   R = (*S*)-PGME
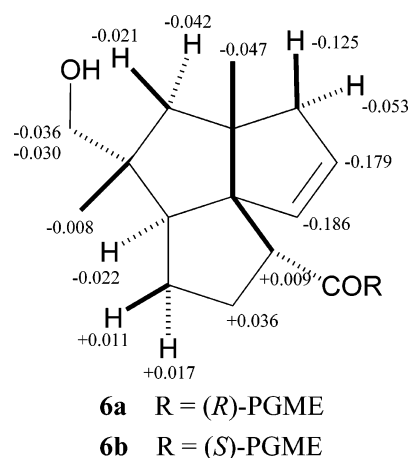
Figure 2. Observed chemical shift differences ($\Delta\delta = \delta_S - \delta_R$, 400 MHz) for the *R*- and *S*-PGME-amides of 12-hydroxysilphinen-15-oic acid (**6**).

found in **2** except that the C-3 methylene protons were coupled to those of the olefin unit. These data were strongly suggestive of a silphinene skeleton.[10,11]

The structure of compound **6** was established by interpretation of HMBC data (Table 3). HMBC correlations from H-9 to C-1, C-4, C-8, and carboxylic acid carbon C-15, together with those from the oxymethylene protons to C-5, C-6, C-7, and C-13 and from H₃-14 to C-3, C-4, C-5, and C-8 indicated that **6** is 12-hydroxysilphinen-15-oic acid. Other correlations observed were fully consistent with this assignment. The relative configuration was assigned by analysis of NOESY data. NOESY correlations between H-9 and H₃-14, H₃-14 and H₃-13, and H₂-12 and H-7 indicated that the carboxylic acid and hydroxymethyl groups both adopt α-orientations, affording a relative configuration analogous to other previously reported silphinene analogues.[10−13]

Determination of the absolute configuration of **6** was attempted using a method developed for chiral α,α-disubstituted propionic acid derivatives.[14] Treatment of compound **6** with (*R*)- and (*S*)-phenylglycine methyl ester (PGME) under suitable amidation conditions produced the corresponding amide derivatives, which were purified by HPLC and analyzed by ¹H NMR. The ¹H NMR chemical shift differences ($\Delta\delta = \delta_S - \delta_R$) between signals for the diastereomeric (*R*)- and (*S*)-PGME amide derivatives are shown in Figure 2. Negative $\Delta\delta$ values are expected for protons that reside on the same side of the PGME amide bond plane as the phenyl group in a given PGME derivative, and positive $\Delta\delta$ values are expected for protons on the opposite side.[14] Interpretation of the results on this basis led to assignment of the *R*-configuration

Scheme 1. Proposed Biosynthetic Formation of Penifulvin B (**2**) from Silphinene Analogue **6**

6
(derived from farnesyl unit
via caryophyllene)[15]
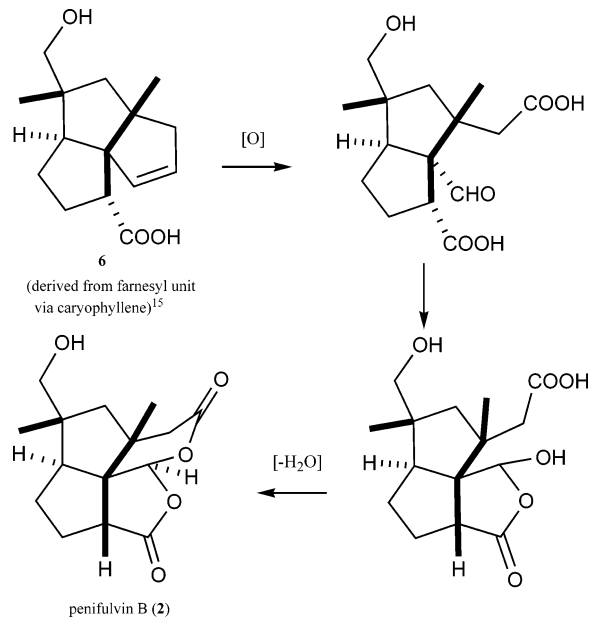
penifulvin B (**2**)

at C-9, and the overall absolute configuration of **6** was therefore assigned as shown.

As noted in the earlier report describing lead compound **1**, penifulvins appear to be biogenetically related to silphinenes. Although silphinenes have been isolated primarily from plants such as *Silphium perfoliatum*,[15,16] silphinene analogues have been previously reported from one other fungal source (phomalairdenones and phomalairdenols; phytotoxins from the blackleg fungus *Phoma lingam*).[12,13] Penifulvins are proposed to be biosynthesized from *E,E*-farnesyl cation via caryophyllene[15] and silphinene intermediates. For example, as depicted in Scheme 1, oxidative cleavage of the C-1−C-2 olefin unit of **6** with appropriate adjustment of oxidation state at C-1 and subsequent bis-lactonization could afford penifulvin B (**2**). Isolation of new silphinene analogue **6** from the same source organism and independent determination of analogous absolute configurations for **5** and **6** as described above support this proposed biosynthetic relationship. The absolute configurations assigned for **1**−**6** are analogous to those of previously reported silphinenes,[16,17] although no absolute configuration has been previously reported for a fungal silphinene.

The two most abundant analogues described here (penifulvin B, **2**; 12-hydroxysilphinen-15-oic acid, **6**) were tested in standard agar disk diffusion assays at 100 μg/disk against *Aspergillus flavus*

(NRRL 6541), *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus* (ATCC 29213), and *Candida albicans* (ATCC 14053) and showed no activity at this level. Most of the antifungal activity of the original extract appears to be due to the presence of the known compound mycophenolic acid. Although the major sesquiterpenoid component penifulvin A (**1**) showed significant activity in an assay against the fall armyworm, *Spodoptera frugiperda*, compounds **2** and **6** were inactive in this assay when tested at similar levels.

Experimental Section

General Experimental Procedures. The optical rotations were measured on a JASCO model DIP-1000 digital polarimeter. NMR spectra were recorded in CDCl$_3$, and chemical shifts were referenced relative to the corresponding signals (δ_H 7.24/δ_C 77.23). ^{1}H NMR data were recorded at 400 MHz (Bruker DRX-400) or 600 MHz (Bruker AMX-600). ^{13}C NMR data were recorded at 100 MHz (Bruker DRX-400). All 2D NMR data were recorded at 600 MHz (^{1}H dimension). HRESIMS data were recorded using a Fisons Autospec double-focusing mass spectrometer. All the HPLC separations were carried out using an Alltech HS Hyperprep 100 BDS C18 column (10 × 250 mm) at a flow rate of 2 mL/min.

Fungal Material. The culture employed in this work (MYC 1728 = NRRL 35584) was originally isolated by D.T.W. from a white mycelial growth on the undersurface of a dead hardwood branch that was collected in a montane dry forest (Ohi'a), Hue Hue Street, Kailua-Kona, Hawaii Co., HI, by D.T.W. in November 2002. General fermentation and extraction procedures employed have been described elsewhere.[4]

Isolation. The crude extract obtained from eight 500-mL fermentation flasks each containing 50 g of rice (1.5 g) was first partitioned between hexanes (3 × 100 mL) and CH$_3$CN (50 mL), and the CH$_3$-CN-soluble portion (0.831 g) was fractioned by silica gel column chromatography using a hexanes−EtOAc solvent gradient. Major component **1** (55 mg) was isolated from a fraction eluted with 95:5 hexane−EtOAc as reported previously.[3] The fraction eluted with 90:10 hexanes−EtOAc (70 mg) was further separated by semipreparative reversed-phase HPLC (30 to 100% CH$_3$CN in H$_2$O over 45 min) to afford an additional sample of penifulvin A (**1**; 9 mg), as well as penifulvin E (**5**; 7.2 mg) and 1-deoxypebrolide (2.0 mg). One additional subfraction from this additional HPLC step was further subjected to semipreparative reversed-phase HPLC (20 to 30% CH$_3$CN in H$_2$O over 45 min) to provide penifulvin D (**4**; 1.0 mg). A fraction from the silica column eluted with 85:15 hexanes−EtOAc (80 mg) was separated by semipreparative reversed-phase HPLC (30 to 100% CH$_3$CN in H$_2$O over 45 min) to afford asperphenamate (35 mg) and a subfraction that was repeatedly subjected to semipreparative reversed-phase HPLC (20 to 40% CH$_3$CN in H$_2$O over 45 min) to afford 12-hydroxysilphinen-15-oic acid (**6**; 2.0 mg). A silica gel column fraction eluted with 80:20 hexanes−EtOAc (20 mg) was separated by semipreparative reversed-phase HPLC (30 to 100% CH$_3$CN in H$_2$O over 45 min) to afford several subfractions, one of which was further subjected to semipreparative reversed-phase HPLC (20 to 30% CH$_3$CN in H$_2$O over 45 min) to afford penifulvin B (**2**; 2.5 mg), penifulvin C (**3**; 1.0 mg), and brevianamide E (1.5 mg). A fraction eluted with 70:30 hexanes−EtOAc (15 mg) was separated by semipreparative reversed-phase HPLC (30 to 100% CH$_3$CN in H$_2$O over 45 min) to afford penifulvin C (**3**; 2.3 mg) and desacetylpebrolide (2 mg). A fraction eluted with 60:40 hexanes−EtOAc (19 mg) consisted mainly of crude brevianamide A, which was identified without complete purification.

Penifulvin B (2): amorphous powder; [α]$^{25}_D$ −87 (*c* 1.2 × 10^{-3} g/mL, CHCl$_3$); ^{1}H NMR, ^{13}C NMR, and HMBC data, see Tables 1−3; NOESY correlations (CDCl$_3$, H-# → H-#) H-1 ↔ H-3α and H-7; H-3α ↔ H-1, H-5α, and H$_2$-12; H-3β ↔ H-5α, H-5β, and H$_3$-14; H-5α ↔ H-3α, H-3β, and H$_2$-12; H-5β ↔ H$_3$-13 and H$_3$-14; H-7 ↔ H-11α and H$_2$-12; H-9 ↔ H-10β and H$_3$-14; H-10α ↔ H-11α and H-11β; H-10β ↔ H-9; H-11α ↔ H-7; H-11β ↔ H-13; H$_2$-12 ↔ H-3α, H-3β, H-5α, H-7, and H$_3$-13; H$_3$-13 ↔ H-5β, H-11β, and H$_2$-12; H$_3$-14 ↔ H-3β, H-5β, and H-9; HRESIMS obsd *m/z* 303.1222 [M + Na]$^+$, calcd for C$_{15}$H$_{20}$O$_5$Na, 303.1208.

Penifulvin C (3): amorphous powder; [α]$^{25}_D$ −140 (*c* 0.92 × 10^{-3} g/mL, CHCl$_3$); ^{1}H NMR, ^{13}C NMR, and HMBC data, see Tables 1−3; NOESY correlations (CDCl$_3$, H-# → H-#) H-1 ↔ H-3α and H-7 and H$_3$-12; H-3α ↔ H-1 and H$_3$-12; H-3β ↔ H-5α, H-5β, and H$_3$-14; H-5α

and H-5β ↔ H$_2$-13 and H$_3$-14; H-7 ↔ H-1, H-10α, H-11α, and H$_3$-12; H-9 ↔ H-10β, H-11β, and H$_3$-14; H-10α ↔ H-11α and H-11β; H-10β ↔ H-9, H-11α, and H-11β; H-11α ↔ H-7, H-10α, and H-10β; H-11β ↔ H-9, H-10α, and H-10β; H$_3$-12 ↔ H-1, H-3α, H$_2$-5, H-7, and H$_2$-13; H$_2$-13 ↔ H$_2$-5, H-10α, H-10β, and H$_3$-12; H$_3$-14 ↔ H-3β, H$_2$-5, and H-9; HRESIMS obsd *m/z* 303.1214 [M + Na]$^+$, calcd for C$_{15}$H$_{20}$O$_5$-Na, 303.1208.

Penifulvin D (4): amorphous powder; [α]$^{25}_D$ −60 (*c* 0.3 × 10^{-3} g/mL, CHCl$_3$); ^{1}H NMR, ^{13}C NMR, and HMBC data, see Tables 1−3; NOESY correlations (CDCl$_3$, H-# → H-#) H-1 ↔ H-3α, H-7 and H$_3$-12; H-3α ↔ H-1 and H$_3$-12; H-3β ↔ H$_3$-14; H-5α ↔ H$_3$-12; H-5β ↔ H$_3$-14; H-7 ↔ H-1 and H$_3$-12; H-10α ↔ H-11α and H-11β; H-10β ↔ H-11α and H-11β; H-11α ↔ H-10α; H-11β ↔ H$_3$-13; H$_3$-12 ↔ H-3α, H-5α, H-7, and H$_3$-13; H$_3$-13 ↔ H-11β and H$_3$-12; H$_3$-14 ↔ H-3β and H-5β; HRESIMS obsd *m/z* 303.1211 [M + Na]$^+$, calcd for C$_{15}$H$_{20}$O$_5$-Na, 303.1208.

Penifulvin E (5): amorphous powder; [α]$^{25}_D$ −80 (*c* 0.5 × 10^{-3} g/mL, CHCl$_3$); ^{1}H NMR, ^{13}C NMR, and HMBC data, see Tables 1−3; NOESY correlations (CDCl$_3$, H-# → H-#) H-1 ↔ H-3α, H-7 and H$_3$-12; H-3α ↔ H-5α and H$_3$-12; H-3β ↔ H-5α and H$_3$-14; H-5α ↔ H$_3$-12; H-5β ↔ H-11β, H$_3$-13, and H$_3$-14; H-7 ↔ H-10α, H-11α, and H$_3$-12; H-9 ↔ H-10α and H$_3$-14; H-10α ↔ H-7, H-9, H-11α, and H-11β; H-11α ↔ H-10α and H-7; H-11β ↔ H-10α and H$_3$-13; H$_3$-12 ↔ H-1, H-3α, and H-7; H$_3$-13 ↔ H-5β, H-11β, H$_3$-12, and H$_3$-14; H$_3$-14 ↔ H-3β, H-5β, H-9, and H$_3$-13; HRESIMS obsd *m/z* 303.1230 [M + Na]$^+$, calcd for C$_{15}$H$_{20}$O$_5$Na, 303.1208.

12-Hydroxysilphinene-15-oic acid (6): amorphous powder; [α]$^{25}_D$ −100 (*c* 0.5 × 10^{-3} g/mL, CHCl$_3$); ^{1}H NMR, ^{13}C NMR, and HMBC data, see Tables 1−3; NOESY correlations (CDCl$_3$, H-# → H-#) H-1 ↔ H-2 and H-7; H-2 ↔ H-3α, H-3β, and H-1; H-3α ↔ H-2 and H-5α; H-3β ↔ H-2 and H$_3$-14; H-5α ↔ H-3α and H$_2$-12; H-5β ↔ H$_3$-13 and H$_3$-14; H-7 ↔ H-11α, H-11β, and H$_2$-12; H-9 ↔ H$_2$-10 and H$_3$-14; H$_2$-10 ↔ H-9, H-11α, and H-11β; H-11α ↔ H$_2$-10 and H-7; H-11β ↔ H-7 and H$_3$-13; H$_2$-12 ↔ H-5α, H-7, and H$_3$-13; H$_3$-13 ↔ H-5β, H-11β, and H$_2$-12; H$_3$-14 ↔ H-3β, H-5β, and H-9; HRESIMS obsd *m/z* 251.1652 [M + Na]$^+$, calcd for C$_{15}$H$_{23}$O$_3$, 251.1647.

(R)- and (S)-MPA Esters of Penifulvin E (5). A solution of **5** (0.5 mg) in distilled CH$_2$Cl$_2$ (500 μL) was treated with (S)-(+)-α-methoxyphenylacetic acid [(S)-MPAOH, 1.0 mg, μmoL], DMAP (one crystal), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 0.5 mg, μmoL). The mixture was stirred at 25 °C for 50 h. One milliliter of H$_2$O was added, and the mixture was extracted with CH$_2$Cl$_2$ (3 × 1.5 mL). The combined organic extracts were concentrated, filtered, and evaporated to give a white solid, which was then subjected to HPLC (30 to 100% CH$_3$CN in H$_2$O over 45 min) to afford (S)-MPA ester **5a** (0.3 mg). Analogous treatment of **5** (0.5 mg) using (R)-MPAOH afforded (R)-MPA ester **5b** (0.2 mg).

(S)-MPA ester 5a: amorphous powder; ^{1}H NMR (400 MHz, CDCl$_3$) δ 5.86 (1H, s, H-1), 5.48 (ddd, *J* = 0.8, 2.2, 6.0 Hz, H-10), 3.03 (br s, H-9), 2.79 (d, *J* = 15 Hz, H-3α), 2.38 (d, *J* = 15 Hz, H-3β), 2.33 (dd, *J* = 4.8, 10 Hz, H-7), 2.12 (m, H-11α), 1.90 (m, H-11β), 1.69 (d, *J* = 14 Hz, H-5β), 1.57 (d, *J* = 14 Hz, H-5α), 1.21 (s, H$_3$-12), 0.85 (s, H$_3$-13), 0.80 (s, H$_3$-14); NOESY correlations (CDCl$_3$, H-# → H-#) H-1 ↔ H-3α, H-7 and H$_3$-12; H-3α ↔ H-1 and H$_3$-12; H-3β ↔ H$_3$-14; H-5α ↔ H$_3$-12; H-5β ↔ H$_3$-13 and H$_3$-14; H-7 ↔ H-1, H-11α, and H$_3$-12; H-9 ↔ H-10α and H$_3$-14; H-10α ↔ H-9 and H-11α; H-11α ↔ H-10α and H-7; H-11β ↔ H$_3$-13; H$_3$-12 ↔ H-1, H-3α, H-5α, H-7, and H$_3$-13; H$_3$-13 ↔ H-5β, H-11β, and H$_3$-12; H$_3$-14 ↔ H-3β, H-5β, and H-9.

(R)-MPA ester 5b: amorphous powder; ^{1}H NMR (400 MHz, CDCl$_3$) δ 5.86 (s, H-1), 5.46 (ddd, *J* = 0.8, 2.6, 6.0 Hz, H-10), 3.12 (br s, H-9), 2.79 (d, *J* = 15 Hz, H-3α), 2.38 (d, *J* = 15 Hz, H-3β), 2.26 (dd, *J* = 5.2, 10 Hz, H-7), 2.19 (m, H-11α), 1.63 (m, H-11β), 1.45 (d, *J* = 14 Hz, H-5α), 1.36 (d, *J* = 14 Hz, H-5β), 1.15 (s, H$_3$-12), 0.93 (s, H$_3$-14), 0.63 (s, H$_3$-13); NOESY correlations (CDCl$_3$, H-# → H-#) H-1 ↔ H-3α, H-7 and H$_3$-12; H-3α ↔ H-1 and H$_3$-12; H-3β ↔ H$_3$-14; H-5α ↔ H$_3$-12; H-5β ↔ H$_3$-13 and H$_3$-14; H-7 ↔ H-1, H-11α, and H$_3$-12; H-9 ↔ H-10α and H$_3$-14; H-10α ↔ H-9 and H-11α; H-11α ↔ H-10α and H-7; H-11β ↔ H$_3$-13; H$_3$-12 ↔ H-1, H-3α, H-5α, H-7, and H$_3$-13; H$_3$-13 ↔ H-5β, H-11β, and H$_3$-12; H$_3$-14 ↔ H-3β, H-5β, and H-9.

Preparation of PGME Derivatives of 6. The PGME derivatives were prepared according to the method of Yabuuchi and Kusumi,[14] using identical reagent concentrations and reaction conditions for the preparation of both sets of derivatives. A sample of **6** (0.7 mg) and 1.0

mg of (*R* or *S*)-PGME hydrochloride (Aldrich) were dissolved in 1 mL of dry DMF. The solution was cooled in an ice−water bath, and 2.0 mg of PyBOP, 1.0 mg of HOBT, and 50 μL of *N*-methylmorpholine were added. The mixture was stirred at room temperature for 7 h. EtOAc (5 mL) was added, and the organic layer was collected and extracted with H_2O (2 × 5 mL). After removal of solvent, 1.3 mg of the crude (*R*)-PGME derivative and 1.7 mg of the crude (*S*)-PGME derivative were obtained in the two reactions. Purification by reversed-phase HPLC (C18, UV detection at 215 nm, 30 to 100% CH_3CN in H_2O over 45 min, 2 mL/min) afforded **6a** (0.3 mg) and **6b** (0.5 mg).

12-Hydroxysilphinen-15-oic acid-(*R*)-PGME-amide (6a): amorphous powder; ¹H NMR (400 MHz, CDCl₃) δ 7.30 (5H, m, phenyl group protons), 6.39 (d, *J* = 6.9 Hz, NH), 5.61 (dt, *J* = 2.0, 5.8 Hz, H-1), 5.50 (d, *J* = 7.0 Hz, C*H*NH), 5.47 (dt, *J* = 2.2, 5.8 Hz, H-2), 3.68 (s, OCH₃), 3.37 (d, *J* = 10.5 Hz, Ha-12), 3.31 (d, *J* = 10.5 Hz, Hb-12), 2.78 (t, *J* = 8.4 Hz, H-9), 2.38 (dt, *J* = 2.2, 17 Hz, H-3α), 2.29 (dt, *J* = 2.2, 17 Hz, H-3β), 2.11 (dd, *J* = 7.8, 11 Hz, H-7), 1.94 (m, H₂-10), 1.92 (d, *J* = 13 Hz, H-5α), 1.56 (d, *J* = 13 Hz, H-5β), 1.38 (m, H-11β), 1.27 (s, H₃-14), 0.98 (s, H₃-13).

12-Hydroxysilphinen-15-oic acid-(*S*)-PGME-amide (6b): amorphous powder; ¹H NMR (400 MHz, CDCl₃) δ 7.29 (5H, m, phenyl group protons), 6.33 (d, *J* = 7.0 Hz, NH), 5.52 (d, *J* = 7.0 Hz, C*H*NH), 5.42 (dt, *J* = 2.2, 5.8 Hz, H-1), 5.29 (dt, *J* = 2.2, 5.8 Hz, H-2), 3.70 (s, OCH₃), 3.28 (d, *J* = 0.5 Hz, Ha-12), 3.33 (d, *J* = 10.5 Hz, Hb-12), 2.79 (dd, *J* = 7.8, 9.0 Hz, H-9), 2.33 (dt, *J* = 2.2, 17 Hz, H-3α), 2.16 (dt, *J* = 2.2, 17 Hz, H-3β), 2.09 (dd, *J* = 8.0, 11 Hz, H-7), 1.98 (m, H₂-10), 1.88 (d, *J* = 13 Hz, H-5α), 1.76 (m, H-11α), 1.54 (d, *J* = 13 Hz, H-5β), 1.39 (m, H-11β), 1.23 (s, H₃-14), 0.97 (s, H₃-13).

Supporting Information Available: ¹H and ¹³C NMR spectra of **2**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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